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Phenotypic and genotypic characterization of antimicrobial resistance in Escherichia coli isolates

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Background: The major influences on the increase and spread of antimicrobial resistant bacteria are the use of antimicrobial agents in human medicine and their use in livestock for therapy and growth promotion. To generate baseline data to use in future risk assessment of antimicrobial resistance, a number of surveillance program on the local, continental and global scale have been initiated. The prevalence of resistance in commensal *E. coli* is a good indicator for the selective pressure by antibiotics use and resistance problems to be expected in pathogenic bacteria. The aim of this study was to determine and compare the occurrence of antimicrobial resistance phenotypes and genotypes among *E. coli* strains recovered from animal sources.

Methods & Materials: Fifty-eight (58) E. coli strains were isolated from animal sources (Cattle, pig and chicken). MIC was determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Strains were screened and mapped by PCR methods for antimicrobial resistant genes, integrons and gene cassettes.

Results: The resistance profile of *E. coli* for ampicillin (100%), cefotaxime (100%), ceftazidime (83%), ciprofloxacin (43%) (C), tetracycline (100%) (T), gentamicin (90%) (G), kanamycin (77%) (K), streptomycin (100%) (S), chloramphenicol (97%) (Ch) sulphamethoxazole (100%) (Su) and trimethoprime (97%) (T). Eight-Five per cent (85%) of *E. coli* harbored class 1 integron and variable gene cassettes were revealed. A prevalence of gene cassettes that present resistance to streptomycin and trimethoprime was observed. The resistance genes determinant present in gentamicin resistance were aac(3')-IIc, aac(6')-Ib and ant(2")-1a. The occurrence of aph(3)-Ia in kanamycin resistant strains was 68%. The prevalence of *sul1* (80%) and *sul2* (100%) was observed. The prevalence of *tet(A)* is higher than *tet(B)* in tetracycline-resistant strains. The gene determinants for chloramphenicol resistance (cat1 and catB) were detected. Differences in the rates and profile of resistance were observed, possibly reflecting differences in antibiotic use regimens among these strains.

Conclusion: This study shows that multi-drug resistant *E. coli* isolates are prevalent in animal sources and a considerable proportion of *E. coli* strains are resistant to a variety of antimicrobial agents. Genotypic characterization revealed high number of diverse resistance determinants.

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New continuous fluorometric assay for bacterial transglycosylase

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Background: The emergence of antibiotic resistance has prompted scientists to search for new antibiotics. Transglycosylase (TGase) is an attractive target for new antibiotic discovery due to its location on the outer membrane of bacteria and its essential role in peptidoglycan synthesis. Though there are few molecules identified as TGase inhibitors in the past thirty years, none of them was developed into an antibiotics for humans. The slow pace of the development is perhaps due to the lack of continuous, quantitative and high-throughput assay available for the enzyme.

Methods & Materials: A new continuous fluorescent assay based on Förster resonance energy transfer, using lipid II analogues with a dimethylamino-azobenzenesulfonyl quencher in the lipid chain and a coumarin fluorophore in the peptide chain was developed. During the process of transglycosylation, the quencher-appended polyprenol is released and the fluorescence of coumarin can be detected.

Results: Using this system, the substrate specificity and affinity of lipid II analogues bearing various numbers and configurations of isoprene units were investigated. Moreover, the inhibition constants of moenomycin and two previously identified small molecules were also determined. In addition, a high-throughput screening using the new assay was conducted to identify potent TGase inhibitors from a 120,000 compound library.

Conclusion: This new continuous fluorescent assay not only provides an efficient and convenient way to study TGase activities but also enables the high-throughput screening of potential TGase inhibitors for antibiotic discovery.

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Comparative in vitro activities of TR-1710 and moxifloxacin against Mycobacterium kansasii

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Background: The currently recommended regimen for treatment of pulmonary M. kansasii infection is isoniazid (INH), rifampin (RIF), and ethambutol (EMB) for 12 – 18 months. RIF is the key agent in this regimen and EMB likely decreases the development of resistance to RIF. It is unclear if INH plays a significant role due